

Role of beta-hydroxybutyric acid in diabetic ketoacidosis: A review

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Abstract – Diabetic ketoacidosis (DKA), a complication of diabetes mellitus, is a severe metabolic disease that often requires intensive treatment. Diagnosis of ketosis associated with DKA can be difficult due to variability in the metabolic state of DKA patients. Recognition of the clinical signs and definitive diagnosis are essential for proper treatment. This article reviews the formation of ketoacids during DKA and the role of β -hydroxybutyric acid in the diagnosis and monitoring of DKA.

Résumé – Rôle de l'acide bêta-hydroxybutyrique dans une acidocétose diabétique : Un compte rendu.

L'acidocétose diabétique, une complication du diabète sucré, est une maladie métabolique grave qui exige souvent un traitement intensif. Le diagnostic d'acidocétose associé à l'acidocétose diabétique peut être difficile en raison de la variabilité de l'état métabolique des patients atteints d'acidocétose diabétique. La reconnaissance des signes cliniques et le diagnostic définitif sont essentiels pour un traitement approprié. Cet article examine la formation de céto-acides durant l'acidocétose diabétique et le rôle de l'acide β -hydroxybutyrique dans le diagnostic et la surveillance de l'acidocétose diabétique.

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Introduction

Diabetic ketoacidosis (DKA) is a severe and life threatening metabolic disease caused by an absolute or relative deficiency of insulin in the body (1). A disease of middle-aged dogs and cats, DKA occurs as a complication of diabetes mellitus (1). The clinical presentation can range from ketotic patients that are eating, drinking, and maintaining hydration on their own to the more common ketoacidotic patients that are dehydrated and have other signs such as vomiting, anorexia, and lethargy (1). The intensity of treatment is therefore variable and depends on the severity of clinical signs and the degree of metabolic derangement. Most DKA patients require intensive, in-hospital treatment.

Pathophysiology

Decreased insulin production by pancreatic beta cells, decreased activity of insulin receptors at the cellular level, or both, are responsible for the abnormal glucose metabolism and resulting

hyperglycemia (1,2). One consequence of this dysregulated glucose metabolism is that glucose transport from serum into the cells is inadequate, leading to cellular starvation (1–3). In order to satisfy its cellular energy requirements and maintain cellular integrity, the body utilizes adipose tissue as the main energy source (1,4). This is a protective mechanism designed to prevent cellular starvation and possibly death (1–4). However, as cellular glucose starvation and adipose tissue utilization for energy continue, major metabolic disturbances occur.

Multiple metabolic pathways are involved in this shift in energy utilization. Hormone sensitive lipase, the activity of which is normally inhibited by insulin, is the main mediator of this process (1–4). This enzyme mediates the degradation of triglycerides and formation of free fatty acids (FFA) (Figure 1) (3). To be utilized as an energy source, FFAs must be transported from the peripheral tissues into the mitochondria of hepatocytes (1,3). Once inside the mitochondria, FFAs undergo beta oxidation which converts them into acetyl coenzyme A (acetyl-CoA) (4). Under normal circumstances acetyl-CoA enters the tricarboxylic cycle (3). To do this, acetyl-CoA first needs to pair with oxaloacetate (3) which is derived from pyruvate during glycolysis. In states of decreased intracellular glucose concentration such as DKA, oxaloacetate will be deficient as it will be preferentially shifted into the gluconeogenesis pathway (3). The oxaloacetate deficiency, when combined with overproduction of acetyl-CoA, will shift the further metabolism of acetyl-CoA towards ketone body formation (3,4).

The cycle of cellular starvation and ketone formation is further fuelled by increasing concentrations of diabetogenic hormones (glucagon, cortisol, growth hormone, and epinephrine) (1–4). As cellular glucose levels decrease, hepatic glycogenolysis

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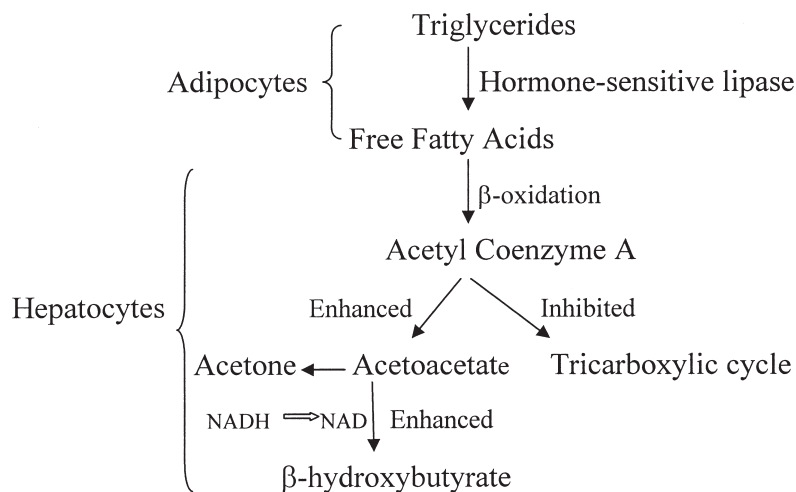


Figure 1. Formation of ketoacids during diabetic ketoacidosis.

increases (1–5). Glycogenolysis is driven by glucagon with the goal being to increase the glucose available to cells. This mechanism is very rapid and occurs within minutes (4). Glucagon acts on the cellular membrane of hepatocytes activating adenylate cyclase which triggers a series of reactions that result in hyperglycemia (4). This series of reactions has the ability to amplify the products of each succeeding step allowing only small amounts of glucagon to stimulate a large-scale glycogenolysis (4). In addition, glucagon acts on hormone sensitive lipase and this indirectly stimulates the release of FFA from triglycerides (3). Glucagon stimulates uptake of FFA by the liver and mitochondrial oxidation of these FFA for energy; once this mechanism is overwhelmed, it promotes formation of ketone bodies (4). One study showed that when excessive amounts of glucagon were given to patients with normal insulin secretion, ketogenesis does not occur (5). Increases in glucagon in conjunction with an inefficient or low insulin level (increased glucagon: insulin ratio), therefore, are needed to amplify ketogenesis (1–5).

Other hormones, collectively called stress hormones, such as growth hormone, epinephrine, and cortisol also play an important role in the pathogenesis of DKA. These hormones inhibit the action of insulin by blocking its receptor sites causing insulin resistance (1–4). Cortisol and epinephrine also stimulate glycogenolysis in the muscles and cause protein breakdown and amino acid release (1,4). These amino acids will be used for further gluconeogenesis in the liver (4).

To be used as an energy source by cells, 2 acetyl-CoA molecules must combine to form 1 molecule of acetoacetic acid (AcAa) and the acetoacetic acid must be carried in the blood to peripheral cells where it can be utilized as an energy source (3,4). Acetoacetic acid is also converted directly to β -hydroxybutyric acid (BHA) and acetone, both of which are ketone bodies. Under normal conditions there is a relatively stable ratio of AcAa and BHA (3,4); however, increased FFA oxidation and acidosis in DKA will lead to reduced mitochondrial redox state (NADH/NAD^+ ratio) which will change the AcAa: BHA ratio in favor of BHA production (3). During DKA, the normal 1:1 ratio of AcAa and BHA is shifted with much higher concentration of BHA being generated (3).

The initial formation of ketone bodies and their utilization as an energy source is a protective mechanism against cellular starvation (1). However, as the overproduction of ketone bodies continues and their utilization for energy decreases due to limited cellular uptake mechanisms, ketone bodies accumulate (2,3). Limited tissue uptake capacity of ketone bodies is primarily mediated by the effective lack of insulin (3). Overproduction of ketones rather than decreased utilization, however, appears to be the primary mechanism of ketone body accumulation during DKA (3–5).

Ketone bodies (primarily BHA and AcAa) are strong acids (1–4). They dissociate freely and produce a large amount of hydrogen ions (1–5). This overproduction of hydrogen ions overwhelms the buffering capacity of the body, quickly leading to metabolic acidosis (1–4). All of the described changes in metabolism contribute to the ongoing state of hyperglycemia, glucosuria, osmotic diuresis, electrolyte changes, ketosis, and acidosis which are seen with DKA (1,4,5).

Diagnosis and monitoring of ketones

The initial presentation of the dog or cat with DKA can vary significantly. Clinical signs can range from polyuria and polydipsia with normal appetite, to decreased mentation, dehydration, and severe metabolic disturbances (1). A subset of patients with ketosis will have been previously diagnosed as having diabetes mellitus (DM) but will not have acidosis and systemic clinical signs other than those of uncomplicated DM (polyuria, polydipsia, and weight loss) (1). Some owners may not have noticed signs of DM and only seek veterinary care for their pet when more serious clinical signs develop. (1–6). The presence of hyperglycemia, ketonemia or ketonuria, metabolic acidosis, glucosuria, and associated clinical signs establish the diagnosis of DKA (1–6); however, making the diagnosis is not as straightforward as the identification of ketones in the urine. When present, the diagnosis is confirmed, but, since not all urine ketones are detected by urine reagent strips, some ketoacidotic patients may be overlooked if the results of urine strips are the only basis for assessing the patient with possible DKA. Ketonuria or ketonemia will be present in patients with DKA. Ketonuria is

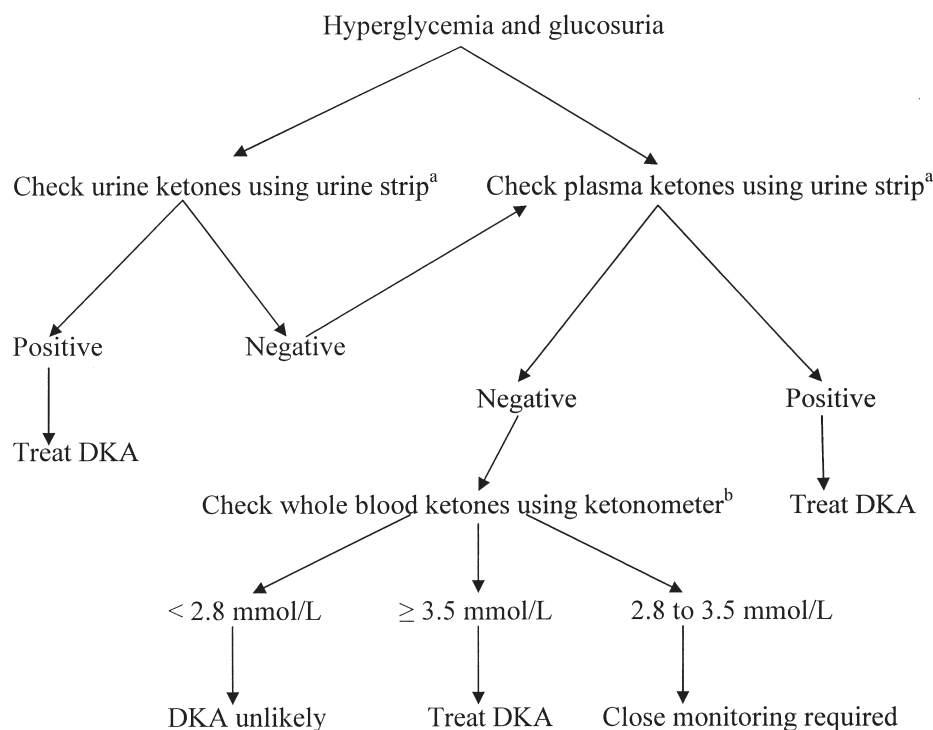


Figure 2. Algorithm for diagnosis of diabetic ketosis.

DKA—diabetic ketoacidosis.

^a Multistix 10SG Urine Reagent Strips; Bayer Corporation, Elkhart, Illinois, USA. Urine and plasma testing methodology utilizes a nitroprusside reaction for acetone and acetoacetic acid detection.

^b Accu-Check Comfort; Roche Diagnostics GmbH, Mannheim, Germany. Whole blood testing methodology directly detects β -hydroxybutyric acid.

most commonly assessed by using commercially available urine reagent strips (Multistix 10SG Urine Reagent Strips; Bayer Corporation, Elkhart, Illinois, USA) (5–8). This methodology relies on a nitroprusside reaction causing a color change as an indicator of the presence of ketones (5–8). Acetoacetic acid and acetone can be detected this way, but not BHA (4–9). Nitroprusside methodology has much higher affinity for AcAa than acetone and since acetone does not contribute to the acidosis, the assumption can be made that urine ketone bodies detected by the nitroprusside reaction are mainly AcAa (3,5–8). False positive results have been reported with this methodology. Medications such as N-acetylcysteine, captopril, and penicillamine can interfere with the testing and give false positive results (3). In addition, as the serum ketone levels increase before urine ketone levels, false negative results were seen when serum AcAa levels were not high enough to reach a renal threshold and be excreted in the urine (3). Urine reagent strips exposed to air for a long time can also show a false negative result (3).

The urine ketone strip test has been modified in order to make the nitroprusside reaction sensitive to BHA by adding hydrogen peroxide (H_2O_2) to the urine of patients suspected of having DKA (9). However, Smith et al (9) showed that although it is possible to convert BHA to AcAa by adding H_2O_2 to the urine containing BHA, the concentration of urine BHA needed for this to show a positive nitroprusside reaction has to be very high. At such high concentrations of BHA, concentration of AcAa in the urine was also high (9). Because of this, addition of H_2O_2 to the urine to enhance the detection of BHA is not

clinically helpful since the concurrently increased AcAa will already be detected via the standard method (9). Therefore, if BHA detection is desired, a direct method for its detection should be used (9).

The same urine reagent strips used to detect urine ketones can also be used to detect ketones in plasma (6,10). Results of one study on cats showed that the sensitivity of the urine reagent strips is higher for ketone detection in plasma than it is for urine (6). The urine reagent strip's response to plasma can be of benefit in the diagnosis of DKA, as patients are often severely dehydrated at initial presentation and obtaining a urine sample may be challenging (1,3). The test is usually performed by adding several drops of plasma from a previously spun hematocrit tube. However, with plasma as with urine, this methodology detects AcAa but not BHA (1,6). As BHA is directly formed from AcAa, and hydrogen ions are required for this reaction, it is expected that the concentration of BHA increases as the animal becomes more acidemic (11,12). Therefore, by using urine reagent strips for ketone detection in plasma, the ketone concentration may be underestimated as the less prevalent type of ketone (AcAa) will be detected in the more severely acidemic animal (11,12). A study evaluating addition of H_2O_2 to the serum did not show any advantage over reported results for urine BHA detection, making the addition of H_2O_2 for better urine reagent strip detection of BHA in plasma not clinically useful (13).

Detection of blood BHA has been used for the last several years when assessing humans with diabetes or DKA (7,14,15).

This has allowed for earlier detection of ketosis and has resulted in earlier treatment. Early detection of DKA in dogs and cats can also have important advantages which range from earlier treatment to better communication with owners.

Because BHA is a predominant ketone body at the onset of DKA, several studies focused on the detection of BHA in the blood of dogs and cats (8,11). A portable point of care ketonometer (PrecisionXtra; Abbot, Alameda, California, USA) developed for human use was evaluated for use with dogs and cats (11). Detection of BHA in blood of dogs and cats using this instrument correlated well with results of BHA detection using an enzymatic method common in reference laboratories (11). Another recent study found that measurement of blood BHA (Accu-Check Comfort; Roche Diagnostics GmbH, Mannheim, Germany) is a more accurate predictor of ketosis than is the detection of acetoacetic acid in urine of dogs (8). The study also found that dogs with a blood ketone concentration ≥ 3.5 mmol/L are at higher risk for developing DKA and that intensive medical management should start if this concentration of blood ketones is detected (8). Dogs that had blood ketone levels < 2.8 mmol/L were unlikely to develop DKA (8).

As many diabetic patients will develop other diseases and may be hospitalized for the treatment of those diseases, close monitoring of blood BHA in these patients can identify patients at high risk for developing DKA. In the past, blood glucose concentrations were primarily used to monitor these diabetic patients. However, it has been shown in humans that the serum glucose concentration does not correlate well with the blood ketone concentration (15). The reason for this is that the rates of glucose and ketone production and utilization are not the same at different stages of the DKA (15). Therefore, severe ketosis can be missed if it is not investigated until severe hyperglycemia is also present (15).

A recent study which surveyed owners of diabetic dogs and cats found that most were willing to perform blood glucose monitoring of their pet at home (16). Since a portable ketonometer is also a glucometer but requires different test strips, blood collection for ketone monitoring should not be any different for the owners who were willing to perform blood glucose monitoring at home. In addition, owners can be educated on when to assess the ketone status of their animal at home and when to seek veterinary care. For example, diabetic animals that develop lethargy, anorexia, vomiting, and urinary tract infection will benefit from close monitoring of BHA. If the BHA blood concentration is > 3.5 mmol/L, or if it is lower but increasing on serial BHA measurements, veterinary care should be sought immediately. This may lead to faster recognition of animals at risk and possible avoidance of progressive ketosis/ketoacidosis and severe clinical signs of DKA. However, as with blood glucose monitoring at home, blood ketone evaluation at home will not be suitable for every owner as a certain skill level will be required for this to be successful. Studies of humans showed much better compliance when a blood ketonometer was used versus urine ketone detection methods for self assessments at home (17,18). However, no veterinary studies investigating owners' compliance for urine testing versus blood testing of glucose or ketones have been published to date.

Monitoring of BHA may be helpful in assessing the efficacy of DKA treatment. In order for peripheral tissues to utilize ketones as energy, insulin has to be present and BHA has to be converted to AcAa (19). Therefore, as DKA is resolving, the concentration of BHA will decrease and the concentration of AcAa will increase (19). By measuring urine or plasma ketones using only the urine reagent strips, the ketosis status of the patient will be overestimated and impending resolution of DKA will not be detected (19). This may lead to the prolongation of intensive treatment and monitoring leading to increased costs. To our knowledge, there have been no veterinary studies done to determine the optimal blood BHA concentration at which intensive treatment of DKA can be stopped. However, if the blood BHA concentration is within normal limits and the patient is eating and drinking well, intensive treatment can be stopped and a change to longer acting insulin preparation can be instituted despite a continued positive urine or plasma ketone test. Until reference values are established, clinicians will have to rely on finding a decreasing BHA concentration concurrently with improvement in clinical signs, acidosis, and other biochemical parameters to determine when to stop intensive DKA treatment.

In conclusion, DKA remains a life-threatening complication of diabetes mellitus and may be detected in newly diagnosed diabetics that have never received insulin therapy. The main factors in the pathogenesis of DKA are effective lack of insulin and a concurrent increase in diabetogenic hormones, especially glucagon (3). Once the disease is suspected, prompt diagnosis and proper treatment are imperative for successful outcome. Recently introduced methods for detection of BHA in blood seem to offer advantages over previously used urine or serum ketone detection methods. Evaluation of the blood BHA concentration in diabetic patients can be useful during any significant concurrent illness. The monitoring of BHA will help the early detection of development of DKA. Increases in blood BHA above 3.5 mmol/L should prompt clinicians to start more intensive treatment (8). Evaluation of the blood BHA of the hospitalized diabetic patient can help to guide treatment and will lead to earlier institution of intensive diabetes therapy if needed. In the future, there may be utility in a low blood BHA concentration indicating less need for intensive treatment or prompting further investigation into the cause of abnormalities such as acidosis presumed to be due to DKA.

Owners of diabetic patients can be trained to evaluate their pet's blood ketone levels. This can be done at home and the owners can be educated as to when to seek veterinary care based on the blood BHA results. This may decrease the occurrence of DKA in the pet population, as studies in humans have shown that blood glucose concentrations do not correlate well with the level of ketonemia at the onset of DKA (15).

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Book Review

Compte rendu de livre

Tasks for the Veterinary Assistant, 2nd edition

Pattengale P. Wiley-Blackwell, Ames, Iowa, USA. 2009. ISBN: 9780-8138-1302-8. 685 pp. \$59.99.

Reading through the pages of this text, I felt transported back to my days in tech school, as the wealth of information serves as an excellent overview of many concepts that form the foundation of work at a veterinary hospital. The chapters are well laid out, and are divided into “Tasks” for each concept, which are even further broken down into step-by-step procedures to ensure the task is completed effectively and efficiently. No details are ever missed in the explanations, and diagrams and figures are found throughout.

There are a number of chapters which go into very good detail in regards to the most important parts of a veterinary assistant's job, such as the section on animal restraint, replete with detailed instructions and images to assist even the most novice animal handler. Concepts involving disease transmission and prevention are not overlooked, and no detail is missed regarding proper cleaning procedures. Some of the more basic veterinary technician skills are also covered, including some laboratory skills, and medication administration.

A few chapters seemed to be a bit excessive on details; the section on knots was brief but very detailed, with more information

on knot types than any one assistant would ever require. The chapter on professional conduct contained quite a few gems, but the sections on personal hygiene (how many times a day to brush one's teeth, bathing, breath mints, etc.) bordered on belittling, and could have been omitted from the text.

The tone of most of the text was a bit harsh, some of the chapter sections came off as a bit strict, and could be off-putting to someone relatively new to the field. Despite the tone, the text is written in a very easy to read way, with a multitude of appendices, including vocabularies and definitions, a number of protocols and general information about a number of species (vitals, vaccination protocols, pain scales, etc.). A competency record is also included for instructors or employers to use along with the text, to make sure students or new hires are learning what they need to.

Overall, the text is an excellent primer for veterinary assistants and new technician students alike. It would make a great addition to any clinic library, or to a college curriculum.

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